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Treatment of Exudative Age-Related Macular Degeneration with Intravitreal Ranibizumab in Clinical Practice: A 3-Year Follow-Up

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Key Words
Age-related macular degeneration · Antiangiogenesis agents · Choroidal neovascularization · Ranibizumab · Vascular endothelial growth factor

Abstract
Purpose: To evaluate the 36-month efficacy of intravitreal ranibizumab injections for choroidal neovascularization secondary to age-related macular degeneration (AMD) in real world clinical practice. ***Methods:*** Retrospective study involving 84 eyes of 77 patients; 52 eyes completed 3 years of follow-up. Subjects were observed initially on a monthly basis and with extended follow-up intervals if signs of quiescence were detected, according to an established protocol. A comprehensive ophthalmologic examination was performed, including best-corrected visual acuity (BCVA) determined with Early Treatment Diabetic Retinopathy Study charts, stereoscopic macular biomicroscopy and optical coherence tomography (OCT) with fluorescein angiography and indocyanine green angiography if considered necessary. Treatment was given if signs of active lesions were present. ***Results:*** The mean baseline BCVA was 49.33 and 49.52 letters at the 36-month visit. The average of treatments was 8.6 at 3 years. At this time point, 77% of treated eyes stabilized or improved their vision (VA loss ≤5 letters). A predictive value for better VA was found for younger age, better baseline VA, good response on OCT and more frequent treatments. ***Conclusion:*** At 3 years, intravitreal ranibizumab is able to maintain baseline VA in exudative AMD patients, with a reduced number of injections, but not to show VA improvement, in clinical practice.

Introduction

Neovascular age-related macular degeneration (nAMD) is a leading cause of irreversible blindness in people ≥50 years old in the developed world [1]. It is expected that, by 2030, age-related macular degeneration (AMD) will be the most important cause of blindness in industrialized countries bypassing diabetic retinopathy or glaucoma.

Ranibizumab (Lucentis®) received the FDA approval in July 2006 and the EMEA approval in January 2007, and is currently indicated for use in nAMD based on the results presented by the phase III studies Minimally Classic/Occult Trial of Anti-Vascular Endothelial Growth Factor Antibody Ranibizumab in the Treatment

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of Neovascular Age-Related Macular Degeneration (MARINA) [2] and the Anti-Vascular Endothelial Growth Factor Antibody for the Treatment of the Predominantly Classic CNV in Age-Related Macular Degeneration (ANCHOR) [3, 4], which showed that monthly intravitreal injection of this drug resulted in visual stability in around 90–95% of treated patients and visual improvement in 1/3 of the cases. Few trials have been published presenting the visual outcomes of anti-vascular endothelial growth factor intravitreal injections in nAMD patients, and different dosing and time regimens were used [5–10]. The long-term efficacy of ranibizumab in the treatment of exudative AMD in real world clinical practice is unknown.

We conducted the present study in order to analyze the 36-month outcomes of intravitreal ranibizumab injections given in an as-needed basis for choroidal neovascularization (CNV) secondary to AMD in clinical practice.

Methods

Study Design

A retrospective, nonrandomized institutional study was conducted. Consecutive patients' medical records were identified by the nAMD diagnosis from June 2006 to March 2009, and the following data were collected: age, gender, date of nAMD diagnosis, best-corrected visual acuity (BCVA) at each visit, fluorescein angiography (FA) and indocyanine green angiography (ICG) at baseline, optical coherence tomography (OCT) data at each visit.

The major inclusion criteria were: (1) age 50 years or older; (2) active primary or recurrent macular neovascularization, whether predominantly classic (type 1 CNV), minimally classic or occult (type 2 CNV), secondary to AMD, juxtafoveal or subfoveal lesions and of all sizes; (3) any BCVA at baseline, and (4) a minimum follow-up of 12 months.

The exclusion criteria were as follows: (1) polypoidal choroidal vasculopathy or retinal angiomatous proliferation (type 3 CNV); (2) secondary choroidal neovascularization not AMD related (high myopia, pseudohistoplasmosis, angioid streaks…); (3) other retinal pathologies such as vein or artery occlusions, juxtafoveal telangiectasias, choroidal tumors, familial macular dystrophies, trauma, or intraocular infection or inflammation; (4) retinal pigment epithelium tear or ripping, and (5) any systemic contraindication to anti-VEGF or angiographic dyes. The preexisting cardiovascular, cerebrovascular or peripheral vascular conditions were relative exclusion criteria.

Eligible patients underwent a comprehensive ophthalmologic examination at baseline, including BCVA determined with Early Treatment Diabetic Retinopathy Study charts (ETDRS), stereoscopic macular biomicroscopy, OCT (Stratus OCT™, Carl Zeiss Meditec, Dublin, Calif., USA, or Cirrus OCT, Cirrus™ HD-OCT, Carl Zeiss Meditec), FA and ICG (Retinal Camara TRC-50IX; Topcon Medical Systems Inc., Oakland, N.J., USA). The patients were monitored each 4 weeks with BCVA evaluation, ophthalmoscopy and OCT (central macular thickness, CMT). The angiographic evaluation (FA and ICG) was done in case of discrepancy between the clinical and morphological data.

The major efficacy end points were the VA change from baseline, the change on OCT-CMT measurements and the number of injections required over the 36-month period. OCT-CMT and BCVA changes were correlated with the following potential risk factors: age, baseline BCVA, baseline angiographic lesion types, number of treatments, presence of subfoveal serohemorrhagic retinal pigment epithelium detachment.

When different OCT systems were used during the follow-up, a correction factor was introduced adding 60 μm to the Stratus's values before analyzing OCT results [11].

Treatment Protocol

Intravitreal injections of ranibizumab were administered to all patients at baseline. No 'loading dose' (3 consecutive monthly injections) was given.

Retreatment was recommended if: (1) new or persistent fluid was demonstrated on OCT; (2) an increase in CMT from previous lowest measurement due to fluid on OCT; (3) VA deterioration related to fluid on OCT, or (4) new or persistent signs of CNV activity on fundus color photography, FA or ICG, including new hemorrhages, leakage on FA and/or early and late hyperfluorescence on ICG.

A flexible strategy was adopted with additional reinjections administered according to the 'retreatment criteria'. If clinical signs remained quiescent for 3 consecutive follow-up visits (no VA changes and dry macula), the intervals were extended to 8 weeks. If the stability was maintained after 6 months, the follow-up intervals were extended to 12 weeks.

Stratus or Cirrus OCT devices were used to evaluate the presence of fluid in the macula and identified as intraretinal fluid (cysts), subretinal fluid or fluid under the retinal pigment epithelium (pigment epithelial detachment).

Statistical Analysis

Statistical analysis was performed using the software PASW Statistics (SPSS, version 18.0, SPSS Inc., Chicago, Ill., USA).

We evaluated the applicability of the assumptions of parametric tests, including normality of distributions by the Shapiro-Wilk test and homogeneity of variances by Levene's test, having verified the applicability of that statistical method for each situation, and when the assumptions were not met we applied the nonparametric equivalent test. For numerical variables we used the ANOVA/independent samples t test or their nonparametric equivalents. The within-eye comparison for the mean VA letter scores and the CMT measurements from baseline values was done using the paired-samples t test or the Wilcoxon signed-rank test. The influence of the baseline FA lesion types on the number of injections over 24 months was assessed using a one-way analysis of variance and the Kruskal-Wallis test. Outcome comparison between different groups was performed with Pearson's χ^2 test or Spearman's correlation coefficients, and relative risk quantification was made with the odds ratio test, respecting the rules of Cochrane. Statistical significance was defined as $p < 0.05$. The data were analyzed from all the patients enrolled in the study until their last VA and OCT findings. The censored or missing cases were handled with the last observation carried forward method.

Table 1. Summary of demographic and baseline characteristics of the study patients (n = 84)

Sex	
Male	35 (41.7%)
Female	49 (58.3%)
Age, years	
Mean	77.39
Range	61–94
CNV type, %	
Predominantly classic	32.1
Minimally classic	22.6
Occult with no classic aspect	45.2
Previous therapy for AMD	
None	67 (80.0%)
PDT	17 (20.2%)
Other pathologies	
Epiretinal membrane	3 (3.6%)
PDR	2 (2.4%)
Glaucoma	5 (6.0%)
Follow-up, months	
Mean ± SD	34.3 ± 6.9
Range	14–48

PDT = Photodynamic therapy; PDR = proliferative diabetic retinopathy.

Results

Demographic Features

The study included 84 eyes of 77 patients, 49 women and 35 men, with an average age of 77.39 years (range, 61–94) followed up for 34.3 months (SD 6.9). Of the 84 eyes, 77 eyes attained 2 years of follow-up and 52 eyes 3 years of follow-up. With respect to the baseline angiographic lesion types, we found that 32.1% patients had predominantly classic, type 2 lesions, 22.6% minimally classic and 45.2% occult, type 1 lesions (table 1).

VA and OCT Changes

At baseline, the mean BCVA was 49.33 letters (SD 15.17). At month 12, the improvement in mean and median VA scores compared to the baseline were +1.64 letters (SD 12.8) and +1.0 letters, respectively. At month 24, the remaining 77 eyes showed a mean and median VA score compared with baseline of –1.25 (SD 16.95) and +2.0. At month 36 of follow-up, the 52 eyes had a mean and median VA score compared with baseline of –1.65 (SD 18.7) and +1.0.

On the first visit, 14 eyes (16.7%) had BCVA <35 letters (20/200) and 10 (11.9%) had BCVA >70 letters (20/40). After 1 year of follow-up (n = 84), 14 eyes (16.7%) gained at least 3 lines of vision (15 letters), 56 eyes (66.7%) experienced stabilization (visual loss of less than 5 letters), whereas 6 eyes (7.14%) experienced BCVA loss of more than 15 letters. After 2 years of follow-up (n = 77), 10 eyes (13.0%) increased BCVA by 15 letters, 51 (66.2%) remained unchanged, whereas 9 eyes (11.7%) experienced BCVA loss of more than 15 letters. After 3 years of follow-up (n = 52), 8 eyes (15.4%) had an increased BCVA of ≥15 letters; 32 (61.5%) experienced stabilization of BCVA loss, whereas 8 eyes (15.4%) experienced BCVA loss superior to 15 letters (table 2).

BCVA changes were not statistically significant in the Wilcoxon signed-ranks test (VA 0–12 months, $p = 0.271$; VA 0–24 months, $p = 0.893$) and paired-samples test (VA 0–36 months, $p = 0.526$). BCVA changes by year are depicted in table 3.

The OCT-CMT changes during the follow-up, however, did achieve the significance with a mean value of 373.30 μm (SD 102.67) at baseline, 296.33 μm (SD 68.57) at 12 months ($p < 0.001$, paired samples test), 259.35 μm (SD 67.93) at 24 months ($p < 0.001$, Wilcoxon signed-ranks test) and 264.28 μm (SD 67.68) at 36 months of follow-up ($p < 0.001$, paired samples test; table 3).

Correlation analyses between the change in OCT-CMT and VA measurements were performed at different time points to analyze the predictive value of the OCT measurements. There was a statistically significant correlation between the OCT-CMT measurements and the VA changes at month 24 (Spearman correlation, $\rho = -0.25$ and $p = 0.02$) and 36 (Spearman correlation, $\rho = -0.38$ and $p = 0.04$). The overall improvement in VA was associated with a decrease in CMT.

Vision loss was defined as a loss of at least 5 letters from the baseline at each time point. Thus, 28 eyes (33.3%) lost vision at 1 year of follow-up, 26 eyes (36.4%) at 2 years and 20 eyes (38.5%) lost vision at 3 years of follow-up.

Vision loss was attributable to formation of subretinal fibrosis, progression of the underlying dry AMD or geographic atrophy with gradual vision loss. Seventeen patients (20%) finished with a disciform scar, the mean time for that occurrence being 22 months.

Treatment Evaluation

The mean and range number of ranibizumab injections in the first, second and third years of follow-up was 3.75 (SD 1.19; 1–6 treatments/eye), 2.64 (SD 1.73; 0–6 treatments/eye) and 2.10 (SD 1.86; 0–6 treatments/eye), respectively. Eleven eyes (13.1%) required less than 3 injections at 1 year of follow-up and 1 eye (1.3%) did so at 2 years of follow-up.

Table 2. VA data

	Loss of ≥30 letters	Loss of ≤15 letters	Mean gain letters	Increase of ≥15 letters	VA ≤35 (20/200)	VA ≥60 (20/63)	VA ≥70 (20/40)
Baseline (n = 84)					16.7% (14)	27.4% (23)	11.9% (10)
12 months (n = 84)	1.2% (1)	92.9% (78)	+1.64 [12.80] –45 to 25	16.6% (14)	21.4% (18)	36.9% (31)	13.1% (11)
24 months (n = 77)	5.2% (4)	88.3% (68)	–1.25 [16.95] –60 to 37	13.0% (10)	24.7% (19)	32.5% (25)	16.9% (13)
36 months (n = 52)	7.7% (4)	84.6% (44)	–1.65 [18.70] –60 to 39	15.4% (8)	26.9% (14)	32.7% (17)	17.3% (9)

Figures in parentheses are numbers, those in square brackets are standard deviations.

Table 3. VA, CMT and number of treatments throughout the follow-up (means ± SD)

	Baseline (n = 84)	3 months (n = 67)	12 months (n = 84)	24 months (n = 77)	36 months (n = 52)
VA, letters	49.33 ± 15.17	52.57 ± 15.26	50.72 ± 15.94	48.79 ± 18.79	49.52 ± 19.72
CMT, μm	373.30 ± 102.67	314.22 ± 102.68	296.33 ± 68.56	259.35 ± 67.93	264.28 ± 67.68
Treatments, n	–	1.53 ± 0.53	3.75 ± 1.20	6.35 ± 2.3	8.67 ± 3.3

Sixty-eight eyes (88.31%) needed retreatment during the 2nd year of follow-up and 35 eyes (67.3%) did so during the 3rd year.

Risk Factor Analysis

There was a statistically significant correlation between the baseline VA and the final VA ($p < 0.001$, Pearson correlation $r = 0.482$). Twenty-three percent of the final VA is explained by the VA at baseline. We found that the worse final VA was correlated with the worse VA at baseline (VA <35 letters; $p < 0.001$, independent samples t test). The better final VA (VA >70 letters) was statistically related with a younger age ($p = 0.024$) and a better baseline VA ($p < 0.01$, independent samples t test).

We studied the good (mean change ≥+15 letters) and bad evolution (mean change ≤–15 letters) according to different possible risk factors. The influence of the baseline VA in the VA evolution was tested. The good evolution at 12 months was statistically related with the lower VA at baseline ($p < 0.006$, Mann-Whitney U test), but the baseline VA seems to have lost importance thereafter.

The influence of baseline angiographic lesion types on the BCVA changes during the follow-up was also analyzed using a one-way parametric analysis of variance. None of the baseline angiographic subtypes were found to have any prognostic impact on the visual outcomes ($p > 0.05$, ANOVA; fig. 1). However, we found a statistically significant difference between these groups in the OCT-CMT changes, with a better outcome of the predominantly classic membrane type ($p = 0.05$, Kruskal-Wallis test; fig. 2). Similarly, no differences were found in the VA baseline parameters and visual outcomes between treatment-naive eyes (66 out of 84 eyes) and previously treated photodynamic therapy eyes (17 out of 84 eyes; $p > 0.05$, Student's t test).

Another risk factor identified for significant loss of BCVA was the presence of a subfoveal serohemorrhagic pigment epithelial detachment. Eight eyes presented with a serohemorrhagic pigment epithelial detachment, 4 of which had significant loss of BCVA at 36 months. This finding was not significant in a Pearson χ^2 test ($p = 0.275$); however, there is a tendency toward a worst evolution in this group with a relative risk of 2.42.

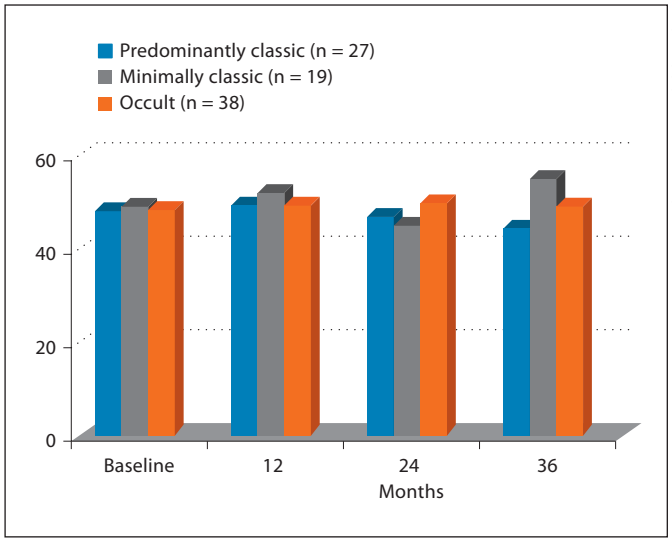

Fig. 1. BCVA evolution (letters ETDRS) in different baseline lesion subtypes.

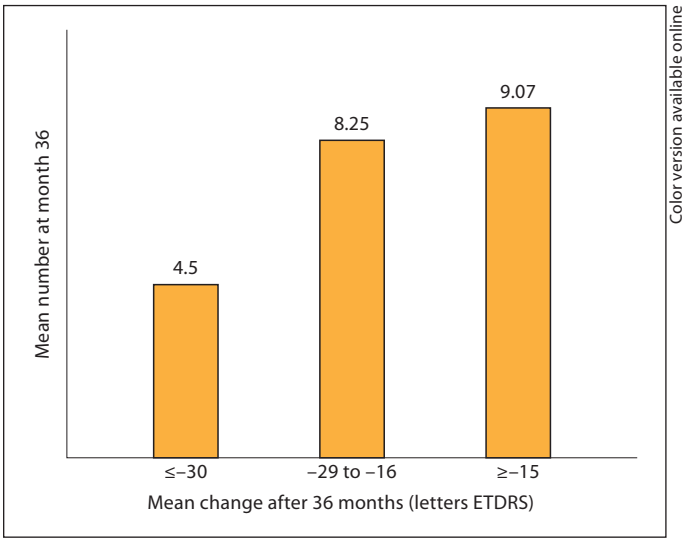

Fig. 3. Number of reinjections and VA change (ETDRS) at 36 months of follow-up: 7.7% of eyes lost ≥30 letters, and 84.6% lost ≤15 letters.

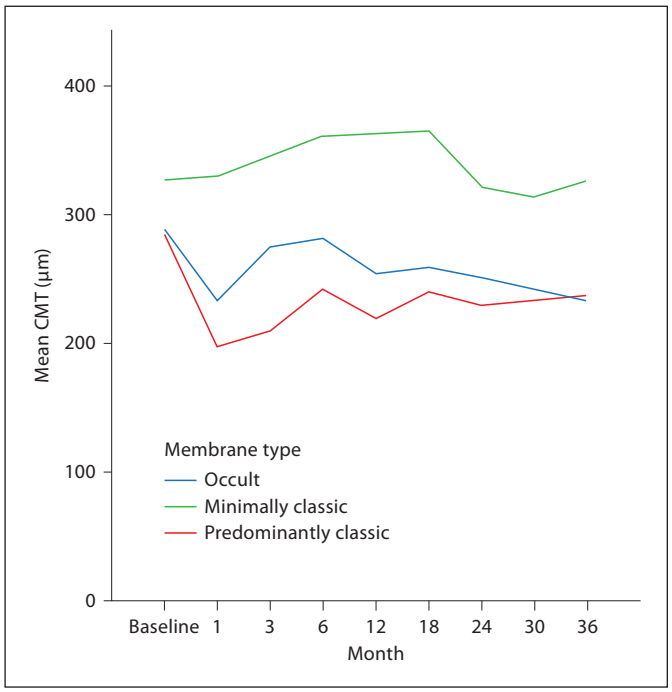

Fig. 2. Mean CMT (µm) evolution during the follow-up among CNV subtypes.

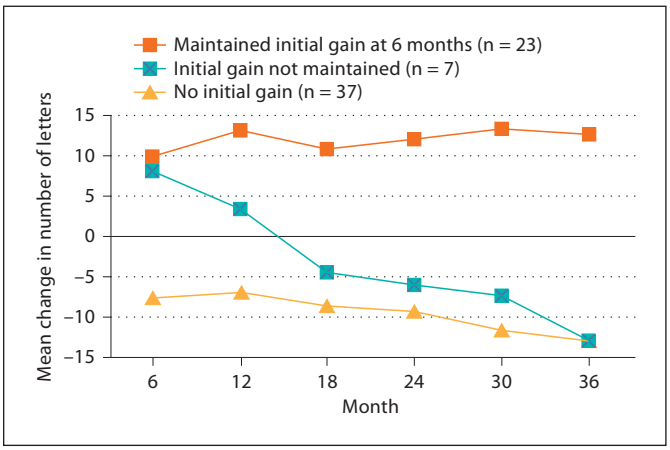

Fig. 4. Evolution of BCVA (letters) in 3 different response groups.

The influence of the number of injections in the VA outcomes was assessed using a nonparametric correlation analysis. A correlation was found between a higher number of injections and a better VA at 36 months of follow-up ($p = 0.028$, Kruskall-Wallis test; fig. 3).

We analyzed the pattern of VA evolution of treated patients and found that 39.3% of them had a VA gain at 6 months that was maintained during the follow-up period; 16.7% showed an initial gain that was not maintained, and 44% of the patients had no initial gain. These patterns of evolution were used for statistical analysis (fig. 4). No statistically significant differences were detected between these groups of clinical evolution in the mean patient's age, baseline angiographic lesion types, baseline VA, baseline CMT measurements or number of treatments performed.

Table 4. Results of nAMD studies

Study	Study design	Study arms	Efficacy end points			Treatments
			mean change VA from baseline/3 months, letters	loss ≥15 letters % patients	gain ≥15 letters % patients	
MARINA (24 months time point) [2]	IVR monthly for 24 months (n = 716)	0.3 mg 0.5 mg sham	+5.4 +6.6 –14.9	8 10 47	26 33 4	24
ANCHOR (24 months time point) [3, 4]	IVR monthly for 24 months (n = 423)	0.3 mg 0.5 mg PDT	+8.1 +10.7 –9.8	6 4 36	34.3 41.0 6.3	24
PIER (24 months time point) [11]	LD + quarterly IVR (n = 184)	0.3 mg 0.5 mg sham	–2.2 –2.3 –21.4	21.8 18.0 41.3	15.0 8.2 4.8	
EXCITE (12 months time point) [12]	LD + quarterly IVR IVR monthly (n = 482)	0.3 mg 0.5 mg 0.3 mg	+4.9/–1.8 +3.8/–2.5 +8.3/+0.8	6.7 8.5 5.2	14.2 17.8 28.7	5.7 5.5 11.4
PrONTO (24 months time point) [16]	LD + PRN (n = 37/40)	n.a.	+11.1	2.5	43.0	9.9
SUSTAIN (12 months time point) [14]	LD + PRN (n = 513)	n.a.	+3.6	7.5	19.3	5.7
SAILOR (12 months time point) [13]	LD + PRN (n = 2,378)	n.a.	+0.5 to 2.3	14.6–19.3	N/A	3.9–4.6
Gupta et al., 2010 (24 months time point) [5]	TER (n = 28/76)	n.a.	+10	4 (12 months)	32 (12 months)	15.91
Marques et al., 2012 (36 months time point)	PRN (n = 52/84)	n.a.	–1.65	11.7	15.8	8.67

IVR = Intravitreal ranibizumab; PDT = photodynamic therapy; LD = loading dose of 3 monthly injections; n.a. = not applicable; TER = treat and extend regimen.

Safety and Adverse Effects

There were no serious ocular events considered to be potentially related to intravitreal ranibizumab treatment as endophtalmitis, uveitis, vitreous hemorrhage or retinal tears in a total of 631 injections performed. No traumatic lens damage was reported, albeit 2 patients changed their phakic status during the follow-up and 13 were already pseudophakics. No long-term effect on intraocular pressure was seen. One 78-year-old patient experienced a nonfatal stroke 1 month after the seventh injection. Another 81-year-old patient suffered from a cavernous sinus thromboembolism 6 months after the fifth injection. One patient died during the second-year study but the death was not deemed to be a drug-related adverse event.

Two, 12 and 4 patients withdrew from the study after the first, second and third years of follow-up, respectively, because of inability to travel or because they refused more treatment.

Discussion

The present study aims to clarify the 36-month outcomes of intravitreal ranibizumab injections given in an as-needed basis and frequent monitoring for CNV secondary to AMD in the real-world clinical practice. Moreover, it was our intention to identify some potential prognostic factors that could help us in the better management of each patient. As far as we know, this is the first study describing the 3-year results of intravitreal

Table 5. Results of nAMD studies

Study	VA, letters/NT		
	12 months	24 months	36 months
MARINA [2] (IVR, 0.5 mg)	+7.2/12	+6.6/24	
ANCHOR [3, 4] (IVR, 0.5 mg)	+11.3/12	+10.7/24	
HORIZON [18] (extension period)	+4.1	+2.0/3.6	
PrONTO [15, 16]	+9.3/5.6	+11.1/9.9	
Gupta et al., 2010 [5]		+10/15.91	
Cohen et al., 2009 [6]	+0.7/3.79		
Marques et al., 2012	+1.64/3.75	−1.25/6.36	−1.65/8.67

IVR = Intravitreal ranibizumab; NT = number of treatments.

ranibizumab injections for AMD patients in clinical practice.

Several studies have been developed to understand which was the best dosing regimen of ranibizumab in order to attempt VA gains compared to MARINA [2] and ANCHOR [3, 4] with fewer treatments. The PIER study [12], the EXCITE study [13], the SAILOR study [14] and the SUSTAIN study [15], all of them showed poorer results when compared to MARINA or ANCHOR. Indeed, the best results with a nonmonthly regimen were obtained in SUSTAIN in which the patients were evaluated every month, suggesting that the efficacy outcomes could be maintained with flexible regimens using a frequent monitoring. They have reported a gain of +3.6 letters with a mean injection rate of 5.7 at 12 months of follow-up. Favorable outcomes were also achieved in the small PrONTO Study [16, 17], a prospective study that used a variable-dosing regimen of intravitreal ranibizumab based on OCT findings to treat nAMD patients, called PRN (pro re nata) regimen. In this trial, a mean visual gain similar to that obtained in MARINA and ANCHOR (table 4) was obtained. After the CATT study [18], there is a belief that treating with ranibizumab every 4 weeks might indeed be equivalent to a treatment 'as needed', providing an average of 7–8 injections in the first year. These kinds of regimen need, however, a close follow-up with frequent visits to monitor the response to treatment and to treat the recurrent exudation [18]. Some small series have been published, in which a 'treat and extend' regimen was tried, allowing a reduction of office visits, with variable results. In this regimen, monthly injections were performed until resolution of signs of exudation, with the treatment interval sequentially lengthened by 1–2 weeks or reduced in case of recurrence of exudation. A study from Gupta et al. [5] of 76 eyes reported good results, comparable to fixed regimens, with a lower mean number of office visits (table 4).

With the increase in the number of diagnosed cases of exudative nAMD and the long disease evolution, it is difficult to maintain a regular monthly follow-up because of frequent constraints including missing appointments and overbooked clinics.

In our study, 85% of patients avoided a 15-letter VA decrease at 3 years of follow-up, compared to 97.5% of such loss in the PrONTO trial and 96% in the treat and extend regimen performed by Gupta et al. [5], both studies with 24 months of follow-up. In our study, only 13.0% patients gained at least 15 letters of VA at 24 months, compared to 26–40% in MARINA/ANCHOR, 43% in the PrONTO study, and 15.8% of the eyes gained ≥15 letters at 3 years of follow-up. When comparing the proportion of patients with 0 or more letters gained at 12 months, we have 56.0% and MARINA and ANCHOR revealed 71.3 and 78% patients with such gains (table 4). In the present study, at 12 months, the rate of eyes that stabilized vision (<5 letters lost) was 66.7% and this was maintained at 24 months (66.23%) and 36 months (61.54%). Similar results were presented by Cohen et al. [6] that showed an improvement of only +0.7 letters (vs. +1.64 letters in our study) at 1 year of follow-up with a mean of 3.79 (SD 1.39) injections (table 5), similar to the 3.75 (SD 1.2) injections performed in our study during the same period. The retrospective analysis included 122 patients and evaluated the visual results of ranibizumab injections at 12 months of follow-up in a clinical setting. 90.3% of the eyes lost fewer than 15 letters and 8% improved by 15 or more letters (compared to 92.9 and 16.6% in our study). Our visual results seem also to be superior to those reported by Rothenbuehler et al. [7], another PRN regimen, that reported 55% of patients avoiding a loss of 15 or more letters at 24 months (vs. 88% eyes in the present study) despite 30% of patients showing an improvement of 15 or more letters (vs. 13%). In fact, clinical settings are quite different from pre-established treatment regimens.

Some reasons have been pointed out to explain these different results. The lack of a loading dose in our study was probably a major contributor to the study outcome. Evidence of that can be demonstrated analyzing the VA improvement pattern during the first 6 months of follow-

up which have been performed here and demonstrated that roughly 40% of the subjects had a VA gain during the first 6 months that was preserved throughout the study period. This raises the question whether a loading dose would increase this effect for those patients or for others who showed no early VA improvement, and whether or not this would have given results closer to those in other studies.

We know from previous already mentioned studies [6, 10] that the poorest outcomes were observed when less than 5 intravitreal injections per year were used and that monthly regular monitoring is required to maintain the efficacy benefits. The retreatment criteria adopted in this trial were derived from the PrONTO study [15] with some differences. In contrast to the PrONTO study, we did not use a cutoff point of 100 μm increase in CMT to treat but treated every increment or persistence of fluid on OCT from previous examinations. However, our results were far inferior to those presented in the PrONTO study. It required strict monthly visits and careful examinations of all the OCT scans in order to detect early signs of recurrence attempting VA loss and maximizing the benefit. According to this, monthly visits should be kept, at least during the first year, and monthly treatment until the retina becomes dry. Despite the desired monitoring on a 4-week interval basis (or extended intervals as recommended by the physician), this was not always achieved, with some patients having a length in follow-ups due to patient constraints or overbooked lists. This lengthy period between follow-ups can be an important cause for the relatively low number of injections per year which could be the major cause for this discrepancy in the VA outcomes.

The influences of VA baseline, age, OCT-CMT response, number of treatments and CNV lesion type on the visual outcomes were also assessed. A predictive value for better VA was found for: younger age, better baseline VA, good response on OCT and frequent treatments. These findings are in accordance with ANCHOR and MARINA studies were it was found that baseline VA, lesion size and age were important predictors of final visual outcomes. Our study reinforces the idea presented in the study of Dadgostar et al. [9] that the visual improvements are related to the frequency of injections received, but also to baseline VA. In fact, our worse results could be partially explained by the probably suboptimal treatment of our patients. Whereas patients in the PrONTO study [15] underwent a mean of 9.9 injections and in the treat and extend regimen study of Gupta et al. [5] 15.91 injections in 24 months, our patients had a mean of 6.35 (SD 2.26, median 6.0) injections during the same 24 months. The number of treatments decreased after the first year from an average of 3.75 (SD 1.20, median 4.0) to 2.64 and 2.08 in the second and third years, respectively. The need for retreatment varied widely among patients and it was unpredictable. The correlation found between the higher number of injections and a better VA outcome could suggest that if the patients were followed up on exactly 4-week periods their outcomes would probably be better.

As proven in this study, a higher baseline VA is also an important factor of good visual prognosis. This fact reinforces the importance of starting treating the patients as early as possible. In our department, patients with exudative AMD have a privileged access to the first injection. This fact allowed us to start the study with a mean baseline VA of 49.33 letters, a value above the baseline VA referred to in other studies in a clinical setting [5, 8]. Nevertheless, at baseline, 16.7% (14 eyes) of our patients had a BCVA <35 letters, and this fact could help to explain our weak results. Having started the treatment with a baseline VA of 49.33 letters we achieve, after 3 years of follow-up and 8.6 injections, a mean VA of 49.52 letters.

Loss of VA and the need for retreatment show that the disease remains active after the first 2 years. In the third year of follow-up, 67.3% of the patients still needed treatment to maintain the macula dry. There is not much information about the 3-year results in AMD patients treated with ranibizumab in clinical practice or in clinical trials. The HORIZON study [19] was an open label extension study in patients who completed the 2-year treatment phase of MARINA, ANCHOR and FOCUS where Lucentis® injections were administered at intervals longer than 30 days according to the investigator criteria. At 3 years of follow-up, after 1 year of PRN regimen, the average gain in VA was +4.1 letters while at 2 years in this flexible regimen the gain decreased to +2.0 letters, thus an average decrease of 7 letters. The +2.0 letters gain was achieved with a mean of 24 injections plus 3.6 in the 24 months of the extension period. The natural progression of the disease allied to the PRN regimen may have had some influence in these long-term results.

We have tried to find out other predictive factors for a better VA outcome. Three different patterns were identified according to the evolution of VA at 6 months: group 1 with VA gain and maintenance (39.3%), group 2 with initial VA gain that was not maintained (16.7%) and group 3 with no initial gain (44%). Although these patterns have been identified at 6 months, it was not possible

to find out risk factors that might identify them previously. In fact, no significant differences were found regarding the mean patient's age, baseline angiographic lesion types, baseline VA, baseline CMT measurements or number of treatments performed.

This study has some limitations and potential sources of bias. The major limitation is the fact that the eyes included in it lack homogeneity. We have included both treatment-naive eyes (66 out of 84) and eyes previously treated with photodynamic therapy (17 out of 84 eyes). This fact did not appear to be significant with no demonstrable statistical differences in terms of visual outcomes between both groups (Student's t test). Also the fact that the study lacks a loading dose is seen as a major limitation that could have masked the true efficacy of PRN treatment. Actually, 7 out of 84 patients did receive 3 injections during the first 3 months but the outcomes were not statistically different (given the small sample together with a small expected effect size). The study has other limitations such as the small sample size, the lack of a control group and the fact that it is a retrospective study, based on clinical records assessment. Also, we realize that 11% of the patients had at least 1 missing appointment and sometimes it was not possible to run a strict 4-week follow-up due to clinical practice constraints. However, this fact was handled in a conservative way, using the last observation carried forward method, in an attempt not to bias the results. Another potential limitation in our trial may be found in VA inclusion criteria. No higher or lower limits were chosen based on the assumption that all included patients could avoid losing initial VA. Patients enrolled with poor vision were unlikely to become any worse and the lower VA provides room for a bigger improvement. The presence of coexisting ocular pathology, as expressed in table 1, and the time to treatment from first diagnosis, which was not considered, may have contributed to our results. In our series, 2 different OCT systems were used. A high proportion of patients had their baseline CMT measured with a Stratus OCT and their final CMT measured with a Cirrus OCT. CMT measures by Cirrus OCT are greater than by Stratus OCT, yet a conversion can be made by subtracting 60 μm from the value obtained with Cirrus OCT [11]. So we have to be careful with the interpretation of our CMT results as we may have introduced some errors.

In conclusion, this study showed that a flexible treatment regimen based on the actual reality of clinical practice is able to maintain baseline VA with a reduced number of injections but results in no VA improvement, at 3 years of follow-up. This is, we believe, the daily clinical practice, where external constraints play a role and partially explain the poor results when comparing with clinical trial results. Doubts remain about whether PRN regimens remain a good choice to treat our patients. These results also support the idea that our current criteria for PRN regimen based on OCT and VA are not good enough to maintain the initial VA gain at 3 years in clinical practice. Attempts to customize treatment in order to limit the number of interventions can actually lead to inadequate treatment and insufficient patient care. It is necessary to find out a different regimen, eventually more customized, able to treat before a VA decline occurs. Ideally, a pattern of retreatment could be found for each patient based on previous behavior which could be periodically adjusted.

Hopefully, further studies will be able to identify and characterize different predictive parameters for visual response to treatment allowing the use of customized treatments while providing the greater benefits.

Disclosure Statement

The authors report no conflicts of interest in this work.

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